On the Shoot Apex of the Cycads*

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The structure of the shoot apex has been and is being widely studied because of its bearing on growth and ultimate origin of the organs and tissues of the shoot. The pioneer investigators in this field traced cell lineage from the base of the shoot apex to the summit as a converging cellular network. Unfortunately both botanists and zoologists thought that the tissues in widely divergent groups could be derived from identically placed apical cells, histogens, or germ layers, as the case might be. Accordingly several theories were proposed to account for cellular configuration and growth in the shoot apex of seed plants. The discovery and wide-spread occurrence of an apical cell in bryophytes and pteridophytes, together with the histologic technique of the times, led several investigators to portray apical cells in both gymnosperms and angiosperms. Subsequent studies, particularly those of Hanstein (1868), revealed the shoot apex as containing the three familiar histogens: dermatogen, periblem and plerome, each of which was perpetuated by a series of superimposed initials. The dermatogen, a uniseriate layer, produced the epidermal system, the periblem of one or more layers, the cortex, and the plerome, the procambium and pith. More recently Schmidt (1924) has interpreted the shoot apex in certain dicotyledons (the theory has been extended to include certain grasses, gymnosperms and species of Lycopodium) as comprised of two zones. The outer, *tunica,* consists of one or more cell layers in which division is periclinal and which as a consequence is characterized by surface growth. The inner zone, *corpus,* is characterized on the other hand by division in all planes and by increase in volume. Still other investigators have considered the shoot apex to be occupied by a primordial meristem characterized by a homogeneous, simple, undifferentiated structure. Further details concerning these theories will be found in two critical surveys by Foster (1939a, 1941a).

The shoot apex in the cycads is of particular interest because its structure can not be interpreted by these proposals and, furthermore, comparative studies of the shoot apex must of necessity include the cycads because of their low position among living seed plants. Representatives from seven of the nine genera have been studied recently. Foster (1939b, 1940, 1941b, 1943) has described and illustrated the structure in *Cycas, Dioon* and *Microcycas* while Johnson (1939, 1944) has investigated *Zamia, Encephalartos, Bowenia* and *Macrozamia.*

Material of *Zamia* may be grown easily from seed or obtained from plants growing in the field in southern Florida. Two-year old plants of *Cycas revoluta*

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or lateral buds which are fairly common on large plants make excellent subjects for study. Fortunate indeed is the botanist who can secure the tip of a really old plant which must be sacrificed for want of space or other reason. Greater difficulty will be experienced in obtaining material from the remaining genera. *Zamia* and *Cycas*, however, will provide a satisfactory picture of the shoot apex as seen in the cycads.

The shoot apex, in mature plants, is deeply sunken and closely invested by a protective armor of leaf bases and cataphylls. These must be removed with considerable care so as not to injure the delicate tip within. The microtechnique outlined by Ball (1941) for the shoot apex will yield excellent preparations.

The apex is characteristically mound-like or broadly cone-shaped in outline and relatively enormous in size. That of *Cycas revoluta* measures 2018-3305 μ in diameter, of *Microcycas calocoma* 500-2000 μ, of *Dioon edule* 1677-1941 μ, of *Encephalartos* 856-1263 μ, of *Bowenia serrulata* 1246 μ, of *Zamia integrifolia* 400-860 μ, and that of an embryo of *Macrozamia Moorii* 625 μ. These dimensions surpass anything reported for other seed plants. Boke (1941) gives 700-900 μ for *Trichocerus spachianus* which appears to be the largest on record among angiosperms.

Median or near-median longsections show that the shoot apex is divided into three or four zones. This condition is indicated schematically in figure 1 and may be observed in the photomicrographs, figures 2 and 3. The summit is occupied by a region of initiation (zone 1); immediately beneath is a core of central mother cells (zone 2) which is underlaid by a rib meristem (zone 4). The three axial zones are surrounded by a series of flanking or peripheral layers (zone 3). Each zone may be distinguished by its contribution to the growth of the shoot as well as by such characteristics as: cell size and arrangement, degree of vacuolation, staining qualities of the nucleus, and thickness of cell walls.

Zone of initiation (zone 1). This zone consists of the relatively small, thin-walled, densely protoplasmic cells in the upper part of the shoot apex.[1] The actual number of cells involved depends upon the size and shape of the apex. In small conical apices there are about a dozen superficial cells, in larger ones from 50 to 100, while in the large dome-shaped apices of *Cycas revoluta* the number will be much higher. There is nothing to suggest an apical cell or even a clearly defined group of initials, for the superficial cells at the summit are indistinguishable from those of zone 3 except possibly by difference in size. Periclinal divisions are sufficiently frequent in both the superficial layer and its derivatives to build somewhat irregular files of daughters which converge toward zone 2 (Fig. 1B). In *Microcycas calocoma* anticlinal and oblique divi-

[1] In two instances I have found large vacuoles in the superficial cells of *Encephalartos*. The specimens were in a dormant condition.

sions at the top "deflect" the files thereby producing a conspicuous fan-like appearance.

Central mother cells (**zone 2**). The central portion of the apex is occupied by a core of tissue roughly spherical, obovoid, cylindrical or fan-like in shape, which is concerned with increase in volume and generation of new cells from its base and sides. Zone 2 originates from the lower part of zone 1, Fig. 1B. Divisions are in all planes but since the daughter cells tend to remain enclosed by the original mother wall a complex arrangement of vertical files and blocks of cells result having the general appearance of a "massiges Meristem." In *Cycas revoluta* vertical files may occur at any level in the central tissue thus making it difficult or impossible to distinguish the central mother cells from the rib meristem. The enlarged mother cells contain thin cytoplasm with large vacuoles and faintly staining nuclei. Their walls are considerably thickened, especially where several cells touch, and display prominent primary pit fields. When the majority of the cells enlarge at one time zone 2 becomes the most conspicuous feature in the apex, (Fig. 2). Following enlargement the mother cells may divide sporadically throughout the zone, while at the periphery they give rise to the rib meristem, (Fig. 1A) and most of the peripheral layers. Apices of *Zamia* which are gorged with starch fail to show a vacuolate central mother cell zone (Fig. 3.)

Peripheral tissue zone (**zone 3**). The zone of initiation and the central mother cells are flanked by this tissue. It is clearly separated into an inner and outer portion. The outer part consists of relatively small, thin-walled, deeply staining, mitotically active cells which owe their origin to divisions in the edge of the initiation zone and to periclinal divisions in the surface layer. The derivatives from the latter may divide in any plane, thereby producing a complex cellular pattern in which cell lineage can not be traced. However, periclinal divisions may predominate and build short files at right angles to the surface (Fig. 1c). Leaf primordia originate from the base of the outer peripheral tissue. The inner layers of the peripheral tissue radiate from and have their origin in the edge of the core of central mother cells. The cells here are larger, more vacuolate and appear to divide less frequently than those in the outer layer.

Growth in the peripheral tissue zone is centrifugal. Epidermis, leaf primordia, cortex, pro-vascular tissue, and generally some of the pith are differentiated from peripheral tissue.

Rib meristem (**zone 4**). The rejuvenated cells at the base of the central mother cell zone build files of daughters which through progressive enlargement ultimately mature as pith. The thickened walls of zone 2 prove to be temporary and do not persist in the rib meristem. The rib meristem is a conspicuous feature in all the cycads examined except in *Cycas revoluta* where a distinction between central mother cells and rib meristem cannot be observed.

The facts presented in this brief survey of the cycad shoot apex, together with the more complete accounts given by Foster and Johnson, suggest the following considerations which are of general morphological interest and possibly may have phylogenetic significance.

None of the seven genera examined show an apical cell in plants old enough to have a well-developed terminal meristem. One-year old seedlings of *Microcycas calocoma* and two-year seedlings of *Cycas revoluta* have already established the growth pattern which is more fully developed in adults (Foster, 1939b, 1943). In *Zamia* the zonal structure was discovered in an embryo prior

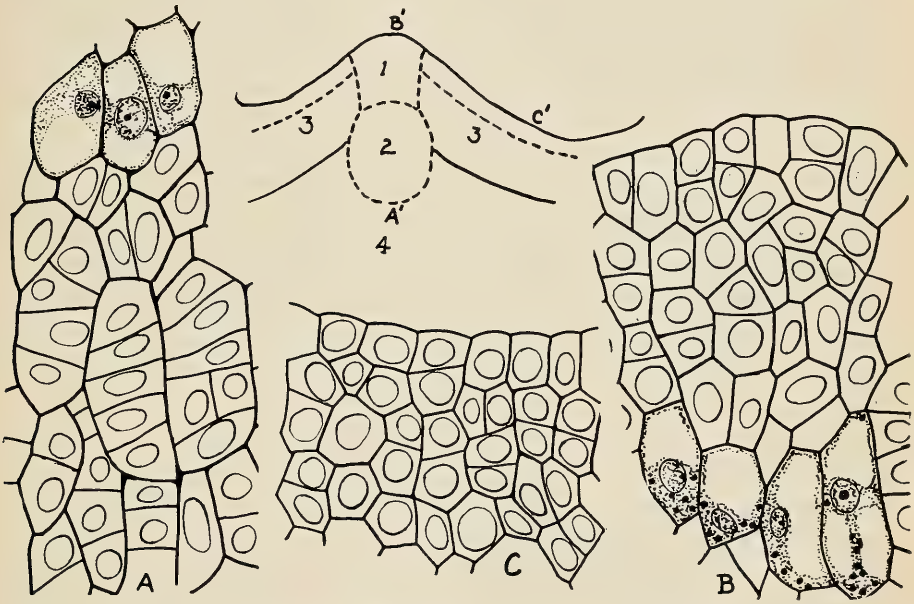

Fig. 1. *Zamia silvicola.* Shoot apex showing zonation: 1, initiation zone, B, detail of same; 2, central mother cell zone, cells containing cytoplasm in B and A are from edge of this zone; 3, peripheral tissue zone, outer portion is derived from zone 1 and the superficial layer as seen in C, inner part is from zones 1 and 2; 4, rib meristem, which originates from zone 2 as seen in A. A, B, and C taken from A', B', C' respectively, × 400.

to emergence from the seed coat. A large cell, in longisection resembling an apical cell, is often seen at the tip of the shoot apex in embryos and seedlings No recent observer, however, has been able to demonstrate that segments are produced from such a cell in the definite sequence required by the apical cell theory. The superficial cells at the exact geometric center of the apex do not differ from their neighbors in either structure or planes of division. Further studies, however, are necessary in the embryology and early growth of the seedling before the possibility of an apical cell can be ruled out.

It is significant that while the apex is divided into zones (Fig. 1), the zones merge more or less and are not sharply delimited as in stratified apices (Figs. 2 and 3). For instance the limits between the initiation zone and the central mother cells fluctuate. The same is true for the central mother cells and the peripheral tissue zone and rib meristem which surround them. The extreme is reached in *Cycas revoluta* where it is impossible to separate the central mother cells, rib meristem and maturing pith. Obviously a histogen theory demanding discrete layers can not be applied to the cycads studied to date. The surface layer continues periclinal divisions throughout the life of the shoot and, therefore, can not be considered as a dermatogen. The concept of a tunica and corpus is likewise untenable. Furthermore the marked zonation definitely precludes considering the apex as a primordial meristem lacking in cellular "differentiation."

Conclusions concerning the similarity of zonation throughout the cycads must remain premature until *Ceratozamia* and *Stangeria* have been investigated. There is, however, a pronounced uniformity in the seven genera studied, if the preliminary account of Johnson (1944) on *Encephalartos, Bowenia* and *Macrozamia* is supported when additional material can be examined. In all, the tissues of the shoot are ultimately derived from the initiation zone. Likewise there is a marked similarity in the central mother cell zones with their large, highly vacuolate, irregularly arranged cells which often have much thickened walls. Upon rejuvenation these cells, with the exceptions noted for *Cycas revoluta,* contribute to the peripheral zone and rib meristem.

The cycad shoot apex furnishes an excellent study in the coordination of growth. Each of the zones increases in size but does not disrupt the general plan or shape of the apex. Growth converges from the initiation zone to the core of central mother cells (a particularly striking feature in *Dioon* and *Microcycas*). The production of new cells accounts for the increase in size in this case. Growth from the central mother cell zone, on the contrary, is divergent with increase in size through cell division and enlargement in the peripheral tissue and young pith cells. A series of actively meristemmatic centers, arranged in the form of a spiral at the base of the apex in *Zamia, Dioon, Cycas* and *Microcycas,* become the leaf primordia. These centers encroach upon the sides of the

Explanation of Figures 2 and 3

Fig. 2. *Zamia latifoliata.* Note: conspicuously vacuolate central mother cells, irregular files of cells in zone of initiation capping the summit, outer part of peripheral zone with files of cells from periclinal divisions and inner part radiating from central mother cells, also rib meristem at the base of central mother cells. × 230.

Fig. 3. *Z. umbrosa.* Shows apex gorged with starch. Note: absence of vacuoles in central mother cells also planes in which division has occurred, and vertical alignment of cells in zone of initiation. × 230.


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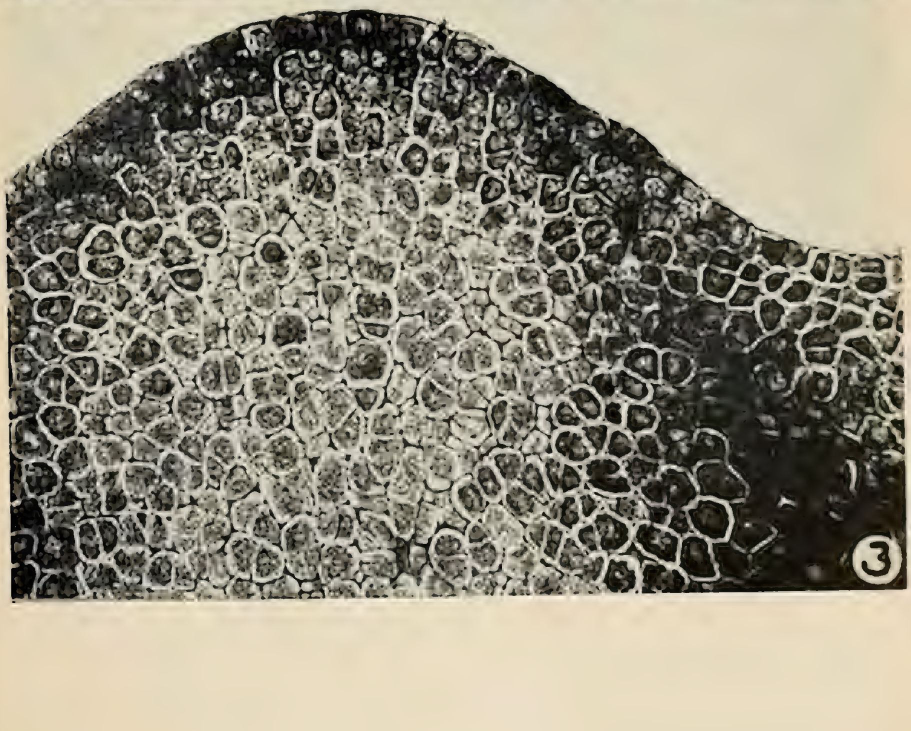
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